Polymer Journal, Vol. 15, No. 5, pp 397—399 (1983)

SHORT COMMUNICATION

β-Form of Poly(α-L-glutamic acid) Induced by Cadmium Ion in Aqueous Solution

Takuzo KUROTU and Motomu KASAGI

Department of Chemistry, The National Defense Academy, Yokosuka 239, Japan

(Received December 15, 1982)

KEY WORDS Poly(α-L-glutamic acid) / Circular Dichroism / β-Form / α-Helix–β-Form Transition /

Interactions between metal ions and polypeptides have been extensively investigated, since complexes formed through these interactions provide useful information on the behavior of metallo-proteins.[1-3] The combination of poly(α-L-glutamic acid) (poly(Glu)) and Cu^{2+} has been utilized as a model system, since it shows an α-helix–random coil transition in a neutral pH region.[4] Other transitions such as the random coil–β-form or α-helix–β-form transition in the presence of other polymers or surfactants have also been studied using poly(L-ornithine)[5] and poly(L-lysine).[6] These transitions, however, have not been investigated in regard to metal ions. The circular dichroism (CD) method is valuable for studying both complex formation and conformational transition of polypeptides or biopolymers.[4] Hence, the conformational change of poly(Glu) induced by cadmium ions as a function of the mixing ratio (Cd^{2+} to Glu residue) or pH was investigated by the CD method.

Sodium poly(α-L-glutamate) was purchased from the Peptide Institute, Inc. (Osaka) (Degree of polymerization, $DP=175$). Reagent grade cadmium sulfate (Wako Pure Chemical Industries, Ltd.) was used without further purification. The pH of all solutions was adjusted with aqueous 0.1 M NaOH or 0.1 M HCl. CD measurements were carried out on a Jasco J-500A spectropolarimeter (Japan Spectroscopic Co., Ltd.) equipped with a DP-500 data processor. The CD spectra were obtained in a quartz cell with a 5 mm path length at a polymer concentration of 0.027 g dl^{-1}. The temperature of the test solutions was maintained at 25°C by circulating water through the cell jacket. The value of f, defined as the ratio of the total molar concentration of Cd^{2+} to the molar residue concentration of polymer, was varied adding appropriate amounts of Cd^{2+}.

In order to clarify the effect of Cd^{2+} on the conformational change of poly(Glu) at various values of f, far UV CD spectra were measured at a fixed pH of 6.68 ± 0.05. The results are shown in Figure 1. The CD spectrum at $f=0$ shows that poly(Glu) in the neutral pH region is in a random coil state characterized by a strong negative band at 196 nm, a weak positive band at 217 nm and a very weak negative band at 238 nm.[7] As f increases from 0 to 0.31 or 1.0, the spectrum undergoes a marked change. The pattern of this spectral change indicates a typical helix–coil transition, in which the α-helix conformation is characterized by a negative band at *ca.* 222 nm associated with the n–π^* transition and another at *ca.* 208 nm associated with the π–π^* transition of the amide group.[7] As f increases from 1.0 to 10.0, the magnitude of the negative residue ellipticity at *ca.* 208 nm decreases gradually and then vanishes, while the CD band at *ca.* 220–222 nm is red-shifted to *ca.* 230 nm. This change in "the Cotton effect" should correspond to the conformational change from α-helix to β-form.[5,8,9] The solution was slightly turbid at $f=10.0$ as a consequence of polymer aggregation.[8,10] Further addition of Cd^{2+} precipitated a small amount of polymer.

The circular dichroism of poly(Glu)–Cd^{2+} complex at $f=10.0$ showed a pH dependence, as evident from Figure 2. Poly(Glu) is in the α-helix conformation in the acidic pH region, but in a random coil

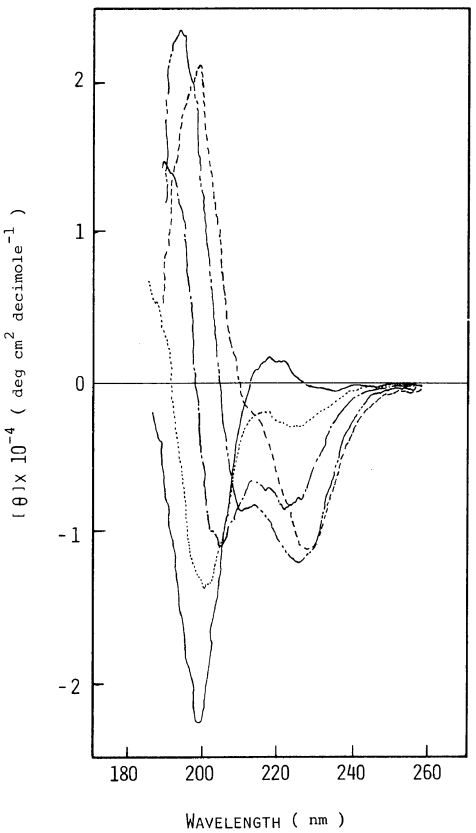

Figure 1. Far UV CD spectra of poly(Glu)–Cd^{2+} solutions at a fixed pH of 6.68 ± 0.05 at different mixing ratios. $f=0$ (no Cd^{2+} present) (——); $f=0.31$ (------); $f=1.0$ (— · —); $f=3.1$ (— — —); $f=10.0$ (— · · —).

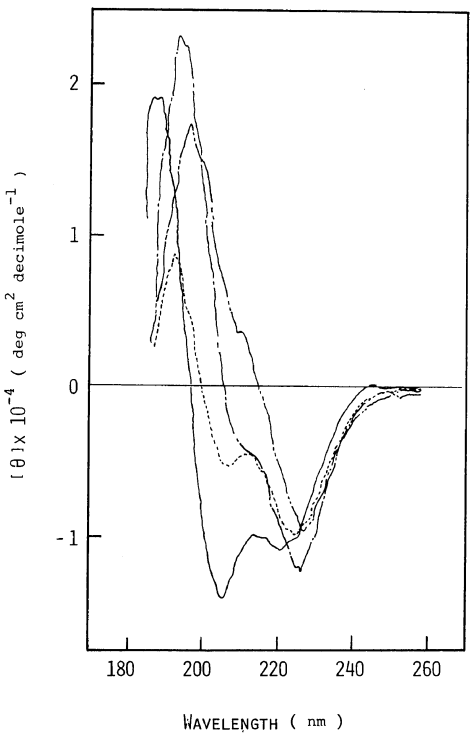

Figure 2. Effect of pH on far UV CD spectra of poly(Glu)–Cd^{2+} solutions at a fixed $f(=10.0)$. pH = 3.81 (——); pH = 5.50 (------); pH = 7.28 (— · —); pH = 7.70 (— — —).

state in the neutral or alkaline pH region.[11] Figure 2 shows that poly(Glu) takes on a α-helix conformation at pH 3.81 and changes from an α-helix to β-form at higher pH. The solution became turbid with increasing pH up to 7.70, while the turbidity due to polymer aggregation disappeared with decreasing pH. The CD spectrum also reversed from the β-form to the α-helix pattern, indicating that the α-helix–β-form transition is reversible.

The above results indicate that the α-helix to β-form transition and the random coil to α-helix transition of the system poly(Glu)–Cd^{2+} depend strongly on the pH and f.

The β-form is considered to consist of an anti-parallel side-by-side arrangement of a polymer strand(s).[12] Therefore, it may be reasonable to assume that Cd^{2+} binds the carboxylate groups in the poly(Glu)–Cd^{2+} complex in various ways, thus, Cd^{2+} bridges the nearest neighbor carboxylate groups on two extended anti-parallel molecular chains, binds the carboxylate groups in extended-parts of a single polymer strand, causing it to take on a hair-pin shape,[13] or binds these groups in both ways. It is, however, not clear at present by which of these complexes the β-form can be explained. In any event, the poly(Glu)–Cd^{2+} complex is a noteworthy example of the α-helix–β-form transition induced by metal ions.

The thermal stability of the β-form was also investigated. The magnitude of residue ellipticity at *ca.* 230 nm for the solution with $f=10.0$ and pH = 7.00 decreased slightly with increasing temperature. The circular dichroism spectrum of this complex was the same as that of the β-form even at the highest temperature examined (80°C). Other ions, Cu^{2+} and Zn^{2+}, which are known to form a complex with poly(Glu), induced no β-form, since complexs with these ions precipitated in the neutral

pH region.

REFERENCES

1. T. Hiraoki, M. Kaneko, and K. Hikichi, *Polym. J.*, **11**, 397 (1979).
2. A. L. Jacobson, *Biopolymers*, **1**, 269 (1963).
3. K. Yamaoka and T. Masujima, *Polym. J.*, **11**, 889 (1979).
4. K. Yamaoka and T. Masujima, *Bull. Chem. Soc. Jpn.*, **52**, 1286 (1979).
5. C. S. Cho, T. Kōmoto, and T. Kawai, *Polym. J.*, **12**, 71 (1980).
6. K. Takeda, A. Iba, and K. Shirahama, *Bull. Chem. Soc. Jpn.*, **54**, 1793 (1981).
7. L. Stevens, R. Townend, S. N. Timasheff, G. D. Fasman, and J. Potter, *Biochemistry*, **7**, 3717 (1968).
8. G. Ebert and Y. Kuroyanagi, *Polymer*, **23**, 1147 (1982).
9. T. Tomiyama and S. Ikeda, *Polym. J.*, **13**, 1111 (1981).
10. G. Ebert and Y. Kuroyanagi, *Polymer*, **23**, 1154 (1982).
11. G. Holzwarth and P. Doty, *J. Am. Chem. Soc.*, **87**, 218 (1965).
12. K. Hamaguchi and H. Takesada, "Tanpakushithu no Senkōsei," Gakkai Shuppan Center, Tokyo, 1971, p 53.
13. H. Maeda and S. Ikeda, *Biopolymers*, **10**, 1635 (1971).